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SOME EFFECTS OF SYSTEMIC N-HYDROXYUREA AND N-HYDROXYURETHANE ON NORMAL AND n-HEXADECANE-TREATED RAT SKIN IN VIVO*

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ABSTRACT

Injections of the two cytotoxic agents, N-hydroxyurea and N-hydroxyurethane, have been shown to increase the normal skin arginase level but to diminish the increase in arginase level of n-hexadecane-treated skin. The effect of the cytotoxic agents on the n-hexadecane-induced hyperkeratotic response was negligible. These selective effects should be useful in experimental dermatology.

The use of n-hexadecane applied to the skin of laboratory animals as a model for studying the hyperkeratinization response has been well documented [1–3]. We have previously reported on the influence of n-hexadecane and other aliphatic hydrocarbons on skin arginase activity and reviewed the literature on this enzyme as a component of skin [4, 5].

N-hydroxyurea and N-hydroxyurethane are effective cytostatic agents [6] with a specific effect on DNA-synthesis [7] and a known effect on hyperplasia of the skin [7, 8]. Hydroxyurea and hydroxyurethane are equipotent on a molar basis [9]. Because a correlation between the soluble arginase activity of the epidermis and hyperkeratinization response is being sought, we believed that the use of N-hydroxyurea or N-hydroxyurethane as specific inhibitors of the arginase response might be instructive. The preliminary findings reported here take no cognizance of morphologic changes nor of the hair-cycle characteristics for rats.

MATERIALS AND METHODS

Materials. N-hydroxyurea (pharmaceutical quality) was supplied by E. R. Squibb & Sons, Ltd., Twickenham, Middlesex, England. N-hydroxyurethane (98% pure) was supplied by Ralph N. Emanuel Ltd., Wembley, Middlesex, England. n-Hexadecane (not less than 99% pure) was supplied by British Drug Houses Ltd., Poole, Dorset, England.

Animals. Female rats derived from the Carworth Farm E strain bred under specific-pathogen-free conditions at this laboratory were used. All rats were aged 12 to 14 weeks and were maintained on Diet 86 (supplied by Scientific Products Farm Ltd., Ash, Kent) and water ad libitum.

Methods. The methods for determining arginase were the same as those described for guinea-pig skin [4] except that the skin slices for rats were 0.3 mm thick and the incubation time was increased from 15 to 30 min. Similarly the epidermis/dermis separations were carried out using 0.3-mm slices of rat skin.

Animal experimentation. Eight groups of 10 rats each were used. The dorsal hair was removed by means of fine electric clippers on the day before any experimentation began. The animals were then treated in groups as follows:

(i) Untreated controls.

(ii) Solvent controls: Animals were injected IP with 0.3 ml of physiologic normal saline. Five hr later, the rats were injected with a further 0.6 ml of saline. Thereafter, 24, 48, 72, and 96 hr after the first injection, further IP injections of 0.3 ml of saline were given. Thirty min after the last injection the rats were killed by cervical dislocation and the skin was removed for estimations to be made.

(iii) Hexadecane controls: n-Hexadecane treatment of skin as in (iv), (vii), and (viii) but with no intraperitoneal injections of cytotoxic agents or solvents.

(iv) Hexadecane controls: As for (ii) but additionally at $24\frac{1}{2}$, $48\frac{1}{2}$, and $72\frac{1}{2}$ hr after the first injection of saline, 2 ml of n-hexadecane was applied to the dorsal skin.

(v) Hydroxyurea controls: As for (ii) but a solution of N-hydroxyurea in saline was injected so that each rat received initially 50 mg then 100 mg followed by 4 more doses of 50 mg N-hydroxyurea.

(vi) Hydroxyurethane controls: As for (ii) but undiluted N-hydroxyurethane was injected so that each rat received initially 0.06 ml (approx. 67 mg), then 0.12 ml (approx. 134 mg) followed by 4 more doses of 0.06 ml N-hydroxyurethane.

(vii) Hydroxyurea and hexadecane: N-hydroxyurea treatment as for (v) plus n-hexadecane treatment as for (iv).

(viii) Hydroxyurethane and hexadecane: N-hydroxyurethane treatment as for (vi) plus n-hexadecane treatment as for (iv).

RESULTS

The mean value for untreated control dorsal skin, 0.3-mm slices, was 12.5 μmole (S.E. ± 3.6) urea released/100 mg dry skin/30 min at 37° C and the epidermis/dermis dry weight ratio 0.28 (S.E. ± 0.05) for 0.3-mm thickness slices. The value for the epidermis/dermis dry weight ratio is in agreement with the value of 0.3 quoted by Cruickshank and Cooper [11], their determinations having been made on hand-cut ear skin slices 0.1 to 0.2 mm thick. Data for the arginase activities and epidermis/dermis dry weight ratios are shown in the Table.

A saline solution of N-hydroxyurea injected IP (Group v) caused a highly significant increase in skin arginase activity; this increase was not significantly different from that caused by application of

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n-hexadecane (Group iv) in these experiments. However, the hydroxyurea did not cause the large increase in epidermis/dermis dry weight ratio associated with the hydrocarbon. Injection of hydroxyurea into rats that were treated topically with n-hexadecane (Group vii) resulted in increased activity when compared with the controls (Group ii) although the increase was not as great as when either material was used alone. The effect on the n-hexadecane-induced hyperkeratotic response was negligible.

Intraperitoneal injection of N-hydroxyurethane (at an equimolar dose to the hydroxyurea used) had no measurable effect on the epidermis/dermis dry weight ratio but caused a significant increase in skin arginase activity (Group vi). This latter effect was significantly less than that observed with hydroxyurea under similar conditions (Group v). The influence of hydroxyurethane injection on n-hexadecane-treated skin (Group viii) was similar to that for hydroxyurea treatment (i.e., a significant diminution in the increase of arginase activity but an insignificant effect on epidermal thickening).

DISCUSSION AND CONCLUSIONS

In earlier studies the authors [4, 5] have established that the relationship of increased skin arginase activity to epidermal thickening under the influence of aliphatic and alicyclic hydrocarbons may be paradoxical. Comparative assessment of arginase activity in different species has been the subject of further studies (Brown and Box; unpublished data). In these, the activity in the skin of rats was found to be less than in the skin of guinea pigs. Other workers [8, 10] have demonstrated that cytotoxic agents, such as hydroxyurea, may have less inhibitory effect on DNA synthesis in normal epidermis than in rapidly proliferating epidermis.

In the study reported here, both hydroxyurea and hydroxyurethane have been shown to increase skin arginase activity in the normal female rat without stimulating the epidermis to thicken to as great an extent (Fig. 1). This differs from the response to topically applied n-hexadecane in which both thickening and increased arginase activity are closely related (Fig. 2).

With n-hexadecane-stimulated skin, the two cytotoxic agents caused a diminution in the in-

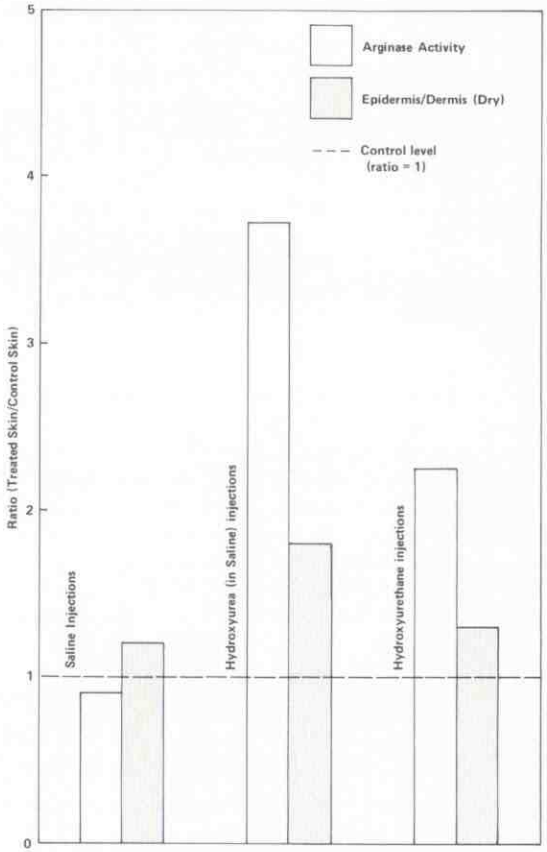

FIG. 1. Effects of injections of saline, hydroxyurea, and hydroxyurethane on the skin of female rats.

TABLE

Summary of responses of female rat skin to topically applied n-hexadecane and/or IP injections of hydroxyurea and hydroxyurethane

Treatment group	Treatment		Mean values ± standard error	
	Skin (topical)	Injection (intraperitoneal)	Epidermis/dermis dry weight ratio	Arginase activity μmole urea/100 mg dry skin/30 min at 37° C
(i)	—	—	0.282 ± 0.050	12.5 ± 3.6
(ii)	—	Physiologic saline	0.348 ± 0.069	11.8 ± 2.4
(v)	—	N-Hydroxyurea in saline	0.523 ± 0.128	46.0 ± 8.0
(vi)	—	N-Hydroxyurethane undiluted	0.373 ± 0.062	28.0 ± 6.0
(iii)	n-Hexadecane	—	0.941 ± 0.175	40.1 ± 5.5
(iv)	n-Hexadecane	Physiologic saline	1.032 ± 0.160	33.8 ± 3.0
(vii)	n-Hexadecane	N-Hydroxyurea in saline	1.160 ± 0.256	25.0 ± 3.0
(viii)	n-Hexadecane	N-Hydroxyurethane undiluted	0.851 ± 0.093	25.0 ± 3.1

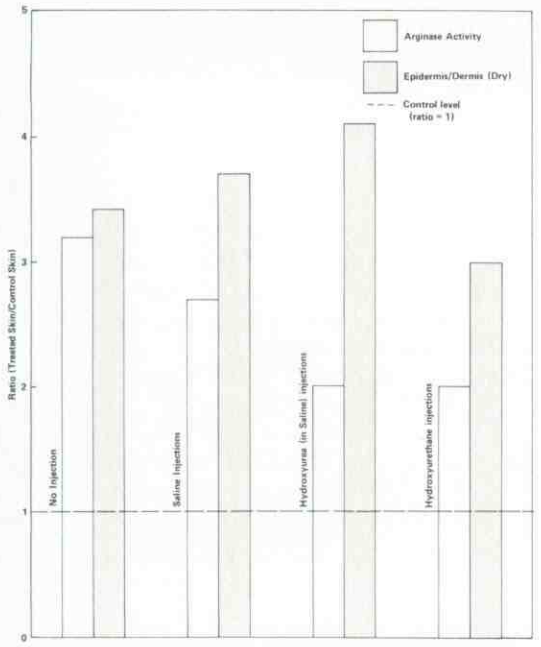

FIG. 2. Effects of injections of saline, hydroxyurea, and hydroxyurethane on the skin of female rats exposed to topical applications of n-hexadecane.

creased arginase activity with no apparent effect on the thickening (Fig. 2).

Discrimination between the epidermal thickening and the increased skin arginase activity has already been observed. This depends on the hydrocarbon applied [5] and it is therefore suggested that the use of either N-hydroxyurea or N-hydroxyurethane in association with measurement of arginase activity and epidermal thickness may be a further experimental tool for investigating the etiology of hydrocarbon-induced dermatitis.

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